

TABLE 7

Feature	Coordinates (nt ##)	Source
pUC 19 ^{*)}	1-191	New England Biolabs (NEB)
Sac I site (unique)	192-197	pUC19
Uptake Sequence	198-212	PCR construct
5' NspA NCR	213-1248	<i>N. mening.</i> , 44-76, PCR construct
Bam H I site	1249-1254	Gent ^r gene cloning site
Gent ^r gene	1255-2104	PCR construct of Gent ^r gene
Sac II (unique)	2105-2110	PCR construct
Rmp promoter 5' fragment (rest)	2111-2230	<i>N. mening.</i> , PCR construct
Mfe I site (unique)	2231-2236	PCR construct
Lac ^q operon	2237-3641	pMAL-p2X (New England Biolabs)
Ptac promoter	3642-3673	pMAL-p2X, PCR construct
Lac operator	3674-3702	pMAL-p2X, PCR construct
RBS	3750-3755	pMAL-p2X, PCR construct
Nde I site (unique)	3761-3766	PCR construct
fHBP (variant 2) leader peptide	3764-3823	<i>N. mening.</i> , 2996, PCR construct
fHBP (variant 2) ORF with stop codon	3824-4588	<i>N. mening.</i> , 2996, PCR construct
SgrAI site (unique)	4589-4596	<i>N. mening.</i> , 44-76, PCR construct
3' NspA and 3' NspA NCR	4597-4638	Previous plasmid for NspA expression
rrnB transcription terminators	4639-4945	pBAD/Thio-E (Invitrogen), PCR
3' NspA NCR	4946-5432	<i>N. mening.</i> , PCR construct
Uptake Sequence	5433-5447	PCR construct
Hind III	5448-5453	pUC19 cloning site
pUC 19	5454-7687 end	NEB, (Amp. ^R)

^{*)}Start from nt. 1 of pUC 19. The plasmid was modified to remove Nde I site for further convenient cloning as follow: It was digested by Nde I - EcoRI and 213 b.p. fragment was removed. Sticky ends were filled in and ligated to restore the plasmid. As a result sited Nde I (183) and EcoRI (395) were destroyed. For cloning of constructs for the expression of target protein we used Sac I and Hind III cloning sites of pUC 19.

[0100] A phase variant of the resulting strain expressing a truncated alpha chain consisting of glucose and galactose. L2 LOS was selected by colony blotting. The resulting genetically modified strain was designated B16B6 HPS-G2, see FIG. 18.

[0101] Strain 44/76 was also modified genetically in the same pattern as described for strain 8570 HOPS-G1. The two

genes, synX and lpxL1, were disabled by insertion mutagenesis, a second porA gene (subtype P1.7-1, 1) was inserted along with its promoter in place of the opaD gene, and a second copy of nadA was inserted behind a porA promoter in place of the nspA gene. Plasmid pBE-311 was used for homologous recombination to insert the NadA gene, the plasmid 3-11 was constructed with the features as described in Table 8 and the sequence can be found in Seq. ID No. 8.

TABLE 8

Feature	Coordinates (nt ##)	Source
pUC19 ^{*)}	1-191	New England Biolabs (NEB)
Sac I site (unique)	192-197	pUC19 cloning site
Uptake Sequence	198-212	PCR construct
5' NspA NCR	213-1248	<i>N. mening.</i> , 44-76, PCR construct
Bam H I site	1249-1254	Gent ^r gene cloning site
Gent ^r gene	1255-2104	PCR construct of Gent ^r gene
Sac II (unique)	2105-2110	PCR construct
PorA promoter (44-76) (modified)**)	2111-3266	<i>N. mening.</i> , 44-76, PCR construct
Nde I site (unique)	3267-3272	PCR construct
NadA (allele 3) leader peptide	3270-3338	<i>N. mening.</i> , 2996, PCR construct
NadA (allele 3) ORF with stop codon	3339-4487	<i>N. mening.</i> , 2996, PCR construct
SgrAI site (unique)	4488-4495	<i>N. mening.</i> , 44-76, PCR construct
PorA terminator (44-76)	4496-4910	<i>N. mening.</i> , 44-76, PCR construct
Bsm I	4911-4916	PCR construct
3' NspA NCR	4917-5329	<i>N. mening.</i> , PCR construct
Uptake Sequence	5330-5344	PCR construct
Hind III	5345-5350	pUC19 cloning site
pUC 19	5351-7584 end	NEB, (Amp. ^R)

^{*)}Start from nt. 1 of pUC 19. The plasmid was modified to remove Nde I site for further convenient cloning as follow: It was digested by Nde I - EcoRI and 213 b.p. fragment was removed. Sticky ends were filled in and ligated to restore the plasmid. As a result sited Nde I (183) and EcoRI (395) were destroyed. For cloning of constructs for the expression of target protein we used Sac I and Hind III cloning sites of pUC 19.

^{**)The 14Gs Poly G tract of the 44-76 promoter was modified by replacing with optimal for the expression 11Gs.}